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During this year we have completed the genotyping and urine analysis for estrogen metabolites and proceeded with the statistical analysis. We have completed two investigations that addressed the following specific aims: 1. To examine the association between polymorphism in genes coding for metabolism and biosynthesis of estrogens (*COMT, CYP1A1, CYP1A2, CYP1B1, and CYP17*) and mammographic densities in healthy women with different ethnic backgrounds. 2. To analyze the relation of mammographic density with the urinary excretion of hormones and the urinary ratio of $2\text{-OHE}_1/16\alpha\text{OHE}1$. As a result of our analyses, 1. We detected that the allele distribution for some genes coding for hormone producing and metabolizing enzymes differed by ethnicity, 2. We found that homozygous carriers of the low activity COMT allele had significantly lower percent mammographic density as compared to subjects with the wild genotype. Subjects with the mutant CYP1A1-exon7-BsrDI and CYP1A1-Msp genotypes, on the other hand, had higher percent densities than women with the wild genotype. 3. We showed that levels of 2-OHE_1, androgens, and total sex steroid hormones were significantly lower in Asian than in Caucasian women. Only the $2\text{OHE}_1/16\alpha\text{-OHE}_1$ ratio was directly related to mammographic densities. None of the individual hormones under study was associated with mammographic densities; therefore, we suggest that the effects of endogenous hormones on breast cancer risk may not be mediated through mammographic densities in adult women. 4. We confirmed our previous findings that Asian women have significantly higher percent densities than in Caucasians. Other factors that affected percent mammographic density were: body mass index, age, menopausal status, parity, age at first live birth before 30, and hormone replacement therapy.

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(4) Introduction

The focus of this study is to identify genetic characteristics that may put women at higher risk to develop breast cancer. Recent research has identified variant alleles in genes that code for common enzymes involved in the biosynthesis and metabolism of endogenous estrogens. In contrast to the low population prevalence of mutations in *BRCA1* and *BRCA2*, variant alleles in these enzyme coding genes are very common (up to 50% prevalence in some populations). Because of their high prevalence, they have the potential to play an important role in breast cancer etiology even if the risk related to these genes is relatively low. It is possible that a large number of women carry variant alleles that predispose them to develop breast cancer. With few exceptions, the polymorphisms investigated in this project have not been examined among women of Asian and Pacific Islander ancestry whose breast cancer risk is lower than among Caucasian women. More than 50% of our study participants are non-Caucasians.

We focused our research on genes involved in metabolism and biosynthesis of estradiol and estrone, the major circulating estrogens in non-pregnant women.

COMT, catechol-O-methyltransferase inactivates catechol estrogens, i.e., 2- and 4-hydroxy compounds. A low $COMT^{LL}$ (Met/Met) and a high $COMT^{HH}$ (Val/Val) activity allele have been identified and the low activity *COMT* allele has been associated with higher breast cancer risk. It has been hypothesized that the low activity *COMT* may be a risk factor for human breast cancer because of its slower inactivation of the potentially carcinogenic 4-hydroxyestradiol. Because of the competing nature of the 2 pathways, an increase in 4-hydroxylation products may lead to a decrease in 2-hydroxylation products. Conflicting evidence with regard to the role of high and low *COMT* in pre- and postmenopausal women has been published.

Estrogen metabolism is partially determined by cytochrome P450 activity and is under the genetic control of both the *CYP1A1* and *CYP1A2* genes. *CYP1A1*, a phase I enzyme, metabolizes a variety of environmental carcinogens and produces many reactive intermediates. It catalyzes the hydroxylation of 17β-estradiol at the C-2 and C-4 positions. Polymorphisms in this gene may influence the degree of 2-hydroxylation. A number of polymorphism have been described in the human *CYP1A1* gene, but research related to breast cancer risk has focused on the *Msp*AI polymorphism.

CYP1A2 plays a major role in the metabolism of many commonly used drugs and may influence the degree of estrogen 2-hydroxylated estrogen metabolites. A recently identified polymorphism was related to increased enzyme activity. A protective effect of a variant allele was described in a recent report.

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CYP1B1 is a key enzyme involved in the production of potentially carcinogenic estrogen metabolites and it appears to be more active than *CYP1A1* in the catalytic efficiency of estradiol hydroxylase. It has its primary activity at the C-4 position of estradiol with a 5-fold lower activity at C-2, whereas CYP1A1 has activity at the C-2, C-16α, and C-15α position. It was suggested that the *m1* polymorphism may be functionally important in breast cancer development, but the few published results are not consistent.

CYP17 gene codes for the cytochrome P450c17α enzyme, located on chromosome 10, spans 6569 bp, divided into 8 exons. It has been proposed that the variant *A2* allele may result in an increased rate of transcription leading to increased estradiol production. The cytochrome P450c17α enzyme mediates both steroid 17α-hydroxylase and 17,20-lyase activities and functions at key branch points in human steroidogenesis. A relation to increased estrogen levels has been described in 2 studies.

The two major innovative features of our project are the simultaneous investigation of several enzyme coding genes and the fact that we examine the relation of genes coding for hormone metabolizing enzymes to probable predictors of breast cancer, urinary hormone excretion, the ratio of different estrogen metabolites, and mammographic densities.

Mammographic density patterns refer to the distribution of fat, connective, and epithelial tissue in the breast and are strong predictors of breast cancer risk. A high percentage of dense parenchyma on mammographic images confers a four- to six fold risk to develop breast cancer (1). Endogenous estrogens and perhaps also androgens are important in the etiology of breast cancer (2-4). An association of mammographic densities and hormone levels is supported by several observations. Hormone replacement therapy increased breast density (5), Tamoxifen treatment improved mammographic densities (6), suppression of ovarian function through a gonadotropin-releasing hormone agonist (7) reduced mammographic densities, and the densities returned to baseline after the treatment was discontinued (8). However, two cross-sectional investigations (9;10) detected no strong relation between mammographic densities and serum estrogen and progesterone levels.

Because some metabolites of endogenous estrogens may have more estrogenic effects than others, we hypothesized that differences in the metabolic pathways may be related to mammographic densities. The metabolism of estradiol follows two major competing pathways, C2- and C16α-hydroxylation, and a minor C4-hydroxylation (11-13). It was proposed that

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women who metabolize a larger proportion of their endogenous estrogen through 16α-hydroxylation are at greater risk because 16α-hydroxyestrone (16α-OHE$_1$) has genotoxic effects, damages DNA, and enhances breast cell growth, whereas 2-hydroxyestrone (2-OHE$_1$) inhibits breast cell proliferation. However, before transformation into methoxy compounds by the enzyme *COMT*, 2-hydroxy compounds also appear to have some estrogenic and growth promoting effects (13). The evidence on the association of the $2/16\alpha$-OH ratio with breast cancer is inconsistent (14-18) and a previous study on mammographic densities observed results contrary to the original hypothesis (19). Women in the highest tertile of the $2/16\alpha$-OH ratio were six times more likely to have a high-risk mammographic pattern. In disagreement with to the hypothesis that women from ethnic groups with lower breast cancer risk have a higher $2/16\alpha$-OH ratio, a comparison between Finnish and Asian women (20) reported a higher $2/16\alpha$-OH ratio in Finnish women.

During this year we completed the genotyping and urine analysis for estrogen metabolites and proceeded with data management and statistical analysis. We performed two analyses that addressed the following specific aims:

1. To examine the association between polymorphism in genes coding for metabolism and biosynthesis of estrogens (*COMT, CYP1A1, CYP1A2, CYP1B1,* and *CYP17*) and mammographic densities in healthy women with different ethnic background.

2. To analyze the relation of mammographic density patterns with the urinary excretion of several hormones and the urinary ratio of 2-OHE$_1$/16αOHE1.

(5) Body

Tasks 1-3 were completed during the first two years and described in previous reports. During this year, we have accomplished the following tasks outlined in the approved Statement of Work.

Task 4. Performing genotyping.

The following genotyping analyses had been completed for 332 study participants: *COMT, CYP17-MspA1, CYP1B1-Pst, CYP1A1-exon7BsrDI, CYP1A1-exon7-Rsa, CYP1A1-Msp,* and *CYP1A2-intron1.* DNA was extracted from whole blood and buccal cells with a rapid method using proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation. The DNA samples were analyzed for the presence of the variant alleles for the genes under study by a PCR/RFLP method. A summary of the analytic methods used to determine polymorphisms is presented in Table 1.

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Task 5. Performing urinary analysis for estrogen metabolites.

Measurements of estrone (E_1), estradiol (E_2), testosterone (T), and 5α-androstane-3α, 17β-diol (ADIOL) concentrations in urine samples were performed with slight modifications from the previously published procedure (21). In brief, 1-ml urine samples were hydrolyzed, purified by solid phase extraction and high performance liquid chromatography, and hormone concentrations measured by radioimmunoassays (RIA) on the dried extracts. All measurements were done in duplicate, including hydrolysis, solid phase extraction, high-performance liquid chromatography (HPLC), and RIA steps. For quality control, two control samples containing known amount of steroids were included for all the analysis steps (from hydrolysis to RIA) in each analytical batch. The detection limits of the method were 2.0 ng/100ml for E_1 and E_2, 7.8 ng/100ml for ADIOL and 1.6 ng/100ml for T. Intra- and inter-batch coefficients of variations were 4.7% and 16%, respectively, for E_1 (at a concentration of 3300 ng/l), 1.7% and 14% for E_2 (at 320 ng/l), 4.7% and 14% for T (at 3300 ng/l), and 7.0% and 11% for ADIOL (at 15.5 ug/l). The metabolites 2-OHE$_1$ and 16α-OHE$_1$ were measured by solid-phase enzyme immunoassays after enzymatic hydrolysis with Helix Pomatia (Estramet, Immunacare Corporation, Bethlehem, USA). Mean intra-batch and inter-batch coefficients of variations are 10% and 15%, respectively, for both analytes.

Task 6. Data management and analysis.

Mammogram Density Assessment. Cranio-caudal views of the mammogram were obtained from the mammography clinics after the radiologic evaluation had been completed and ruled out any malignancy. The films were scanned into a PC using a Kodak LS-85 X-ray digitizer with a pixel size of 260 μm (equal to a resolution of 98 pixels per inch). The principal investigator performed computer-assisted mammographic density assessment using a method developed in Canada (22). The reader first chooses a threshold value that defines the outline of the breast and then selects the best threshold to identify the regions that represent mammographic densities. The pixel count corresponding to the dense area is determined by the computer, as is the total area within the outline of the breast. Percent density was calculated as the ratio of the dense area to the total area of the breast multiplied by 100. A random sample of 58 mammograms was read in duplicate. The intraclass correlation coefficients (23) were 0.95 (95% CI: 0.92-0.97) for the size of the dense areas and 0.98 (95% CI: 0.97-0.99) for the total breast area, and of 0.97 for percent density (95% CI: 0.95-0.98).

Data management. Results of genotyping, urinary analyses, and mammographic density assessment information were entered into the study database. The SAS statistical software package version 8.2 (SAS institute Inc., Cary, NC 1999-2001) was used for data management and statistical analyses. The preliminary examination of the data included an investigation of outliers. We explored the normality of all distributions. If the distribution of a variable deviated strongly from normality, we calculated the natural logarithm to normalize the distributions and to maintain their continuous nature. For some analyses, percent density was classified into five categories: <10%, 10 to 24.5%, 25 to 49.9%, 50 to 74.9%, and 75% and over. Body mass index (BMI) was calculated as the ratio of weight in kilograms divided by the square of the height in meters. For the stratified analysis, subjects were classified into underweight (BMI<18.5), normal weight ($18.5 \leq$ BMI < 25), overweight ($25 \leq$ BMI <30), and obese (BMI > 30) categories, according to the WHO/CDC classification.

Ethnicity was assigned according to the following rules. A woman was classified as Caucasian if both parents had some Caucasian ancestry and shared no other ethnic background. Subjects who reported not more than three ethnic backgrounds were classified as Chinese, Japanese, or Filipino, if both parents were of the same ethnicity or if the mother was of the respective ethnic background and the parents shared no other ethnic background. In agreement with rules applied in the State of Hawaii (24), women with any Hawaiian background were classified as Native Hawaiian. For some analyses, we further categorized women into 3 ethnic groups: Caucasians, Asian (including Chinese, Japanese, and Filipino women for the reason of the similarity in percent densities) and mixed /other category (containing women of mixed ethnic backgrounds that did not fit one of the above groups and Native Hawaiian women because of their mixed ancestries).

Statistical analysis. We considered percent density the most important mammographic density characteristic and used it as the main outcome measure. Analysis of variance was applied to test for associations between genotype and mammographic characteristics while controlling for confounders. Only variables significantly associated with percent density were included into the final model. We also reported the absolute difference in percent density and results of the post hoc t-test to show paired comparisons between variables with more than 2 levels where the difference achieved statistical significance. To show differences in the genotype distribution among ethnic groups, we used χ^2 tests and polytomous logistic regression. We also used

dichotomous logistic regression to obtain the odds ratios for wildtype versus non-wild genotype carriers of the variant alleles in women of different ethnicity.

To explore associations between mammographic density and urinary hormone measurements, we computed Spearman correlation coefficients (r_s) (25;26) and included potential confounders. Then, we applied analysis of variance to test for associations between ethnicity, hormone levels, and mammographic characteristics with adjustment for confounding variables (26). In addition, we computed least-squares means for the urinary hormone levels by category of percent density using the proc glm procedure in the SAS software package (26). Finally, we performed trend tests to investigate a possible relation between the hormones and percent density. We regressed the mean level of the hormones onto the mean density of each of the 5 density categories.

1. Investigation of the Mammographic Densities and Genetic Polymorphisms in Healthy Women of Different Ethnicity

Results

We included 328 women in the investigation of the mammographic densities and genetic polymorphisms. The mean age of the study subjects was 46.8 years (SD 7,84; range, 34.5 – 85.0 years). The majority of women (267; 81,4%) were premenopausal. The ethnic composition was the following: 118 Caucasian, 90 Japanese, 37 Native Hawaiian, 30 Chinese, 13 Filipino, 13 of mixture of 3 ethnicities or more, and 27 of other ethnicity. For some analyses, subjects were categorized into the 3 ethnic groups: Caucasian (118), Asian (133), mixed (71), with mixed or other ethnicity. Almost all study participants spent the major part of their lives in U.S. There were no significant differences among ethnic groups by age, total daily calories intake, parity, maternity age, history of oral contraceptives use, menopausal status, HRT use, and family history of breast cancer in first-degree female relatives. The average body mass index was 25 kg/m^2 with significant differences among ethnic groups (F=4.5, p=0.0002). BMI was highest in Hawaiians (27.9 kg/m^2) and lowest in Chinese (22.8 kg/m^2). In addition, ethnic groups differed significantly by age at menarche (F=4.53, p=0.0127); Caucasian women were significantly older at menarche than Asian women (13.1 versus 12.6 years old, p= 0.0044).

Mammographic percent density by descriptive characteristics. The mean percent mammographic density in the study population was 40.2% (standard deviation 23.0%; range from 0% to 88.8%). Mammographic density differed significantly among ethnic groups (Table 2). Breast density in Caucasian women was the lowest of all groups: Their breast density was 3.8 % (p>0.05) and

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9.1% (p=0.0017) lower than in the Mixed and Asian groups, respectively. After adjustment for potential confounders, breast density in Caucasian women remained significantly lower than in Asians (p=0.0017) and lower than in women of mixed ethnicity (p=0.25). Postmenopausal women had significantly lower percent density than premenopausal women (30.5% vs. 42.3, F=13.6, p=0.0003). Adjusted values of mammographic density for postmenopausal women were lower than in premenopausal women, but not statistically significant. Among the very small number of women currently using hormone replacement therapy (HRT), breast density was unexpectedly lower, compared with those who never used HRT or used it in the past. This difference was not statistically significant after adjustment for other possible predictors of mammographic density. Mammographic density had significant inverse associations with age, BMI, and parity. With every 5-year increase in age, percent density decreased by 3.37%. An increase of one BMI category was associated with a percent density decrease of 1.94% (t =15.44, p<0.0001). A history of breast cancer among first-degree female relatives and age at menarche did not affect mammographic density. An earlier age at first live birth was associated with lower percent density, but was not significant after adjustment for other factors under study. Percent density was also studied in relation to oral contraceptive use, duration of HRT use, total daily fat and saturated fat consumption, percent of fat and saturated fat in diet, and average daily ethanol consumption, but showed no significant associations with any of these variables.

Genotype by ethnicity. The distributions of all studied genotypes fit the Hardy-Weinberg equilibrium, with the exception of *CYP1A1-Msp* (χ^2=5.5; p=0.019). We found significantly different genotype distributions among ethnic groups for the following polymorphic genes: *COMT, CYP1B1-Pst, CYP1A1-Msp*, and *CYP1A1-exon7-BsrDI* (Tables 3 and 3a). Caucasian women had a significantly higher prevalence of *COMT* and *CYP1B1-Pst* mutant alleles than women of all other ethnicities. On the other hand, the prevalence of the *CYP1A1-Msp* and *CYP1A1-exon7-BsrDI* mutant alleles was significantly lower in Caucasians than in women of other ethnicity. For instance, frequencies of the *COMT Val/Val, Val/Met*, and *Met/Met* genotypes were 17%, 51%, 32% in Caucasian subjects, 50%, 40%, 10% in Asian, and 56%, 35%, 9% in women with mixed or other ethnicity. We applied logistic regression analysis to study the associations between genotypes and ethnicities. Tables 4 and 4a present odds ratios (ORs) and 95% confidence intervals (CIs) for associations between variant allele and ethnicity. Caucasian ethnicity and common allele homozygote were used as reference groups (OR=1.00). Among Caucasian women, the odds of being a homozygous or heterozygous carrier of the low activity *Met* allele were significantly higher than in any other ethnic group. The same pattern was

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observed for the *CYP1B1-Pst* genotype; the odds of having a non-wild *CYP1B1-Pst* genotype were higher in Caucasian women than in all other ethnic groups. The difference reached statistical significance in Japanese subjects; the difference was also statistically significant in Chinese and Filipino women, but was restricted only to heterozygous carriers of the mutant *CYP1B1-Pst* allele. The opposite pattern of genotype distribution among ethnic groups was observed for *CYP1A1-Msp* and *CYP1A1-exon7BsrDI* genes. The odds of carrying one or both *CYP1A1-Msp* mutant alleles were much higher in women of all other ethnic groups and were significantly higher in Japanese, Hawaiian, and Chinese subjects than in Caucasian women. The odds of being a heterozygous carrier of the mutant *CYP1A1-exon7BsrDI* allele were significantly higher in Japanese, Hawaiian, Chinese women, and women of mixed ethnicity than in Caucasians. In Japanese, the odds were significantly higher in relation to both hetero- and homozygous carriers. When the combined Asian and mixed ethnicity groups were compared to Caucasians, the odds of being a heterozygote or homozygote carrier of the *COMT Met* allele were significantly lower; ORs for Asian women were 0.3 (95% CI: 0.1-0.5) and 0.1 (95%CI: 0.05-0.2) and for women with mixed ethnicity they were 0.2 (95%CI:0.1-0.4) and 0.1 (95%CI: 0.03-0.2) for heterozygote and homozygote carriers, respectively. Asian women had significantly lower odds of being hetero- (OR= 0.2; 95%CI: 0.1-0.4) or homozygous carriers (OR=0.1; 95%CI:0.03-0.2) of the *CYP1B1-Pst* mutant allele. Asian women and women of mixed/other ethnicity had significantly higher odds of carrying one or both mutant *CYP1A1-Msp* and *CYP1A1-exon7BsrDI* alleles (Table 4a).

Genotypes and mammographic densities. The associations between polymorphic genotypes and percent mammographic density are presented in Table 5. We found that carriers of the *COMT*, *CYP1B1-Pst*, and *CYP1A2-intron1* mutant alleles had lower percent densities than subjects with the wild genotypes. Mean percent density and least squares mean percent density adjusted for confounders were significantly different among the *COMT* common *Val/Val* allele homozygotes than among heterozygotes and homozygote carriers of the rare *Met/Met* allele (p=<0.05). *Met* allele homozygotes had statistically significant lower percent densities than homozygous carriers of the *Val* allele. The absolute difference in means was 8.87% (p=0.015) and, after adjusting for confounders, 8.56% (p=0.007). The differences in mean density between subjects with different *CYP1B1-Pst* and *CYP1A2-intron1* genotypes were not statistically significant. Heterozygote carriers of the rare *CYP1B1-Pst* allele had significantly lower percent density than common allele homozygotes. The absolute difference in means based on the t-test was significant before and after adjusting for confounders: 6.03% (p=0.02) and 4.6% (p=0.0483), respectively. Carriers

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of the *CYP1A1-Msp* and *CYP1A1-exon7BsrDI* rare alleles had at tendency to have higher mammographic densities than common allele homozygotes; although, the difference in mean percent density was significantly different only among subjects with different *CYP1A1-exon7BsrDI* genotypes (p=0.03). This difference was not statistically significant after adjusting for confounders (p=0.23). The absolute difference in means was 14.55% (P=0.01); after adjustment for confounders the difference between means was 8.33 (p=0.1).

Genotypes by mammographic density stratified by ethnicity (Table 6). Homozygous carriers of the *COMT Met* allele had a tendency to have lower means of percent density than common *Val* allele homozygotes in all ethnic groups, but the difference in means did not reach statistical significance. Heterozygous carriers of the rare *CYP1B1-Pst* allele had significantly lower mammographic density comparing with the common *CYP1B1-pst* allele homozygotes, but only in Caucasian women. The absolute difference in mean percent densities was 13.08% and 15.41% (p=0.0003) after adjustment for confounders. *CYP1A1-exon7BsrDI* rare allele hetero- and homozygotes among Asian women had significantly higher mammographic density compared to women with the common allele homozygotes (p=0.03). The same tendency was observed in Caucasian women, but the difference in means was not statistically significant.

Discussion

In our study, we had an opportunity to investigate the distribution of variant alleles for several genes that code for enzymes involved in the biosynthesis and metabolism of endogenous estrogens and the relation of these polymorphisms with mammographic densities among healthy women with different ethnic background. Because of their high prevalence, these low-penetrance genes have a potential to play an important role in breast cancer etiology even if the risk associated with these genes is relatively low.

Studying distributions of the variant alleles in women with different ethnic background, we found that the prevalence of the mutant alleles for several polymorphic genes (*COMT, CYP1B1-Pst, CYP1A1-Msp*, and *CYP1A1-exon7-BsrDI*) differed significantly by ethnicity. We observed that the prevalence of the rare *COMT* and *CYP1B1-Pst* alleles was significantly higher in Caucasian women than in women of all other ethnicities. On the other hand, the prevalence of the variant *CYP1A1-Msp* and *CYP1A1-exon7-BsrDI* alleles in Caucasians was significantly lower than in other ethnic groups. In respect to mammographic densities, Caucasian women had significantly lower breast densities than Asian study participants, and this difference was

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significant after adjustment for age, BMI, menopausal status, HRT use, age at menarche, parity, and age at first live birth. Low mammographic densities were associated with variant *COMT*, *CYP1B1*, and *CYP1A1-intron1* alleles, although only the association between mammographic densities and the low activity *COMT Met* allele was statistically significant before and after adjustment for confounders. Several studies have reported associations between this allele and breast cancer risk, but there has been no agreement to date on the direction of this association (27-30). However, another study that investigated the relation between breast density and the *COMT* polymorphism described similar results as our, i.e., lower densities for carriers of the mutant allele (31). In our study, both Caucasian ethnicity and high prevalence of the *COMT* low activity Met allele were significantly associated with lower mammographic densities. Another interesting finding was related to the heterozygous carriers of the rare *CYP1B1-Pst* allele who demonstrated lower mammographic densities than common allele homozygotes when studied in the whole sample; this association was even stronger in the subgroup of Caucasian women. The *CYP1A1-exon7-BsrDI* variant allele was associated with higher percent densities in the whole study population and in the subgroup of Asian women.

2. Investigation of the Mammographic Densities and Urinary Hormones in Healthy Women with Different Ethnic Background

Results

We included 305 women into the investigation of the mammographic densities and urinary hormones in healthy women with different ethnic background. The majority of the women was premenopausal; only one out of four women was postmenopausal (Table 7). The mean age was 47.2 years and Caucasian women were slightly younger than Asian women. The BMI was lowest among Asian women, intermediate in Caucasian, and highest in the Mixed/Other category (p = 0.0004). Percent density also differed significantly by ethnicity (p = 0.003), even after adjustment for age, menopausal status, and BMI (p = 0.03). Percent density was highest among Asians and lowest among Caucasians, 45.2% vs. 34.9%. Of the 305 women, 35, 48, 108, 87, and 27 belonged to the five density categories. Close to 40% of Caucasians were in the two lowest density categories, whereas only 20% of Asian women were classified that way. Breast density was significantly higher for pre- than for postmenopausal women (44.2 ± 23.0% vs. 28.1 ± 19.2%) The density difference between Caucasians and Asians was greater after menopause (16% vs. 9%).

Excretion of all hormones combined was lowest among Asians and similar in the two other groups, but this difference was not quite statistically significant (p = 0.09). The difference in

hormone levels was primarily due to $2\text{-}OHE_1$, ADIOL, and T, which differed significantly by ethnicity, even after stratification for menopausal status or HRT use. While androgen levels did not vary by HRT use, estrogen levels were approximately twofold higher among postmenopausal women on HRT than among non-users. However, after stratification by menopause and HRT use, androgen levels remained lower among Asian than Caucasian women in each subgroup. On the other hand, we observed similar levels of $16\alpha\text{-}OHE_1$, E_1, and E_2 in the three ethnic groups. The $2OHE_1/16\alpha\text{-}OHE_1$ ratio was approximately 25% lower in Asian women than in the other two groups (p = 0.04). The ethnic difference in the $2/16\alpha\text{-}OHE_1$ ratio was greater before (1.56 vs. 2.11) than after menopause (1.55 vs. 1.72) for Asian and Caucasian women, respectively. The $2\text{-}OHE_1/16\alpha\text{-}OHE_1$ ratio did not differ by HRT use (p = 0.57).

All hormone levels declined with age (Table 8). The correlation coefficients for all hormones combined was $r_s = -0.28$, while the individual correlations varied between $r_s = -0.14$ and -0.39. These relations were strongest for ADIOL and the two estrogen metabolites, followed by T, and weaker for E_2 and E_1. The $2/16\alpha\text{-}OH$ ratio was not significantly related to age. Only the androgen levels were associated with BMI; women with a higher BMI excreted more T and ADIOL. This association did not change after excluding women on HRT. As a result, BMI showed a significant inverse relation with the estrogen/androgen ratio. In postmenopausal women, none of the associations between hormones and BMI was significant, only E_2 was weakly correlated with BMI ($r_s = 0.31$, p = 0.08) among women not using HRT. The association of BMI with androgens was slightly weaker among post- than premenopausal women. Percent density was negatively related with age and BMI, but not with estrogens or androgen levels. A weak positive association with $2\text{-}OHE_1$ disappeared after adjustment for confounders. The correlation between the $2/16\alpha\text{-}OH$ ratio and mammographic density was 0.07 (p = 0.22) after adjustment for confounders. Restricting the correlation analysis to premenopausal women did not change the correlations. After stratifying the small group of postmenopausal women by HRT use, mammographic density was positively related to E_1, both hydroxymetabolites, and testosterone. The correlation coefficients were between $r_s = 0.32$ and 0.38, but did not reach statistical significance. However, among postmenopausal women on HRT, E_1 and both hydroxymetabolites were negatively associated with percent density with correlation coefficients of $r_s = -0.39$.

Mean levels of hormones by categories of percent density (Figure 1) illustrate the lack of an association of breast density with E_1, E_2, $2\text{-}OHE_1$, T, and ADIOL. The p-values for the respective trend tests were 0.94, 0.85, 0.54, 0.78, and 0.69. For $16\alpha\text{-}OHE_1$, we observed a weak negative

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relation (p for trend = 0.19) that translated into a 25% higher 2/16α-OH ratio for women in the highest density category. The respective values for the five categories of percent density were: 1.59, 1.68, 1.86, 1.85, and 1.99 with a p-value of 0.01 for the linear trend test. Excluding the 37 women on HRT changed this trend only minimally. However, stratification by menopausal status showed that the association was stronger in pre- than postmenopausal women (p = 0.08 vs. 0.97 for postmenopausal). HRT use did not affect the relation among postmenopausal women. Separate analysis for women with a BMI of 25 or less (p = 0.07) vs. women with a BMI greater than 25 (p = 0.40) indicated that the relation was restricted to women with normal weight. Stratification by ethnicity showed similar trends in Caucasian and in Asian women (p = 0.07 and p = 0.04), but the mean 2/16α-OH ratio was lower for Asians than for Caucasians in all density categories.

Discussion

In this cross-sectional investigation among women of different ethnicity, we observed higher percent densities, lower urinary androgen levels, and a lower 2/16α-OH ratio among Asian than Caucasian women. However, we did not observe any significant associations between urinary hormone levels and mammographic density. The 2/16α-OH ratio showed a relation with percent densities in a direction opposite to our initial hypothesis. Women with percent densities of 75% or greater had an approximately 25% higher 2/16α-OH ratio than women with percent densities below 10%. This relation was similar in Asian and Caucasian women, but it was not present among postmenopausal. The ethnic differences in breast density agree with previous studies (32;33). Due to the smaller breast size of women with Asian ancestry, percent density is higher than in Caucasian women. However, the absolute size of densities appears to be lower among Asian women (34).

Our findings agree with the only previous investigation of mammographic density and the 2/16α-OH ratio (19). That study among postmenopausal women used a qualitative assessment method of mammographic density assessment. The mean 2/16α-OH ratio was 1.12 in the high-risk group and 0.83 in the low risk group, a 35% difference. The results also agree with the higher 2/16α-OH ratio in Finnish women as compared to Asian women who have the lower breast cancer risk (20). Although a number of studies have investigated the 2/16α-OH ratio and breast cancer, the evidence does not offer a definite answer to the question whether a higher 2/16α-OH ratio reduces or increases breast cancer risk. An association with postmenopausal breast cancer was detected in one small case-control study (14), but a larger study in California did not support the

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hypothesis (35). Two cohort studies (17;18) found a higher $2/16\alpha$-OH ratio associated with a non-significantly reduced breast cancer risk among premenopausal women. The most recent study (16), which was conducted among Chinese women, reported a reduced breast cancer risk with a higher urinary $2/16\alpha$-OH ratio, but only when urine was collected prior to breast cancer treatment.

(6) Key Research Accomplishments

1. We detected differences in the allele distribution by ethnicity for the following genes that code for hormone producing and metabolizing enzymes: *COMT, CYP1A1-Msp, CYP1B1-Pst, CYP1A2-Intron*, and *CYP1A1-exon7-BsrDI*. Among Caucasian women, the odds of being a homozygous or heterozygous carrier of the low activity *COMT Met* allele and the *CYP1B1-Pst* mutant allele were significantly higher than in any other ethnic group. The opposite pattern of genotype distribution was observed for the *CYP1A1-Msp* and *CYP1A1-exon7BsrDI* genes. The odds of carrying one or both mutant alleles were significantly higher in Asian women and women of mixed and other ethnicity.

2. In our study, homozygous carriers of the low activity *COMT* allele had significantly lower percent mammographic densities than subjects who had the wild genotype. Carriers of the mutant *CYP1B1-Pst* allele had lower percent densities, but the difference did not reach the statistical significance. Subjects with the mutant *CYP1A1-exon7-BsrDI* and *CYP1A1-Msp* genotypes, on the other hand, had higher percent densities than women with the wild genotypes. Percent density was significantly higher in subjects with the *CYP1A1-exon7-BsrDI* genotype. These findings suggest that mammographic density, a marker of breast cancer risk is associated with the presence of variant alleles for several genes that code for hormone producing and metabolizing enzymes.

3. We found that levels of 2-OHE$_1$, androgens, and total sex steroid hormones (estrone, estradiol, 2-OHE$_1$, 16α-OHE$_1$, testosterone, androstanediol) were significantly lower in Asians than in Caucasians. Only the 2OHE$_1$/16α-OHE$_1$ ratio was directly related to mammographic densities. None of the individual hormones under study was associated with mammographic densities; therefore, it appears that the effects of endogenous hormones on breast cancer risk may not be mediated through mammographic densities in adult women.

4. The study of mammographic densities in women of different ethnicities confirmed our previous findings that Asian women have significantly higher percent densities than Caucasians. Other factors that effected percent mammographic density were: BMI, age, menopausal status, parity, age at first live birth, and HRT use. Age at menarche, family history of breast cancer, history and duration of oral contraceptives use, total calories intake, total calories from fat and saturated fat, daily fat and saturated fat consumption (in grams) were not associated with percent mammographic densities in this study.

(7) Reportable Outcomes

Publications

Maskarinec G, Williams AE, Rinaldi S, Kaaks R. Mammographic densities and urinary hormones in healthy women with different ethnic backgrounds. Hormonal Carcinogenesis IV: Proceedings of the Fourth International Symposium edited by JJ Li (In press).

Published abstracts

Maskarinec G, LeMarchand L. No relation between CYP17 polymorphism and mammographic density. Cancer Epidemiology, Biomarkers, and Prevention 2002; 11:1222s.

Presentations at conference

Maskarinec G, LeMarchand L, Kaaks R. An Investigation of Mammographic Densities, Urinary Estrogen Metabolites, and COMT, CYP1A1, and CYP1B1 Gene Variants in Premenopausal Women (Poster). Molecular and Genetic Epidemiology of Cancer, Kona, HI 1/2003.

Maskarinec G, LeMarchand L. Genetic Polymorphisms, Estrogens, and Breast Density (Poster). Era of Hope 2002 Breast Cancer Research Program Meeting, Orlando, FL, 9/2002.

(8) Conclusions

Future analyses will include the question of interactions between different polymorphisms although our sample size is rather limited. The finding of lower mammographic densities among carriers of the low activity COMT enzyme requires further investigation and the search for plausible mechanisms of action.

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(10) Appendices

Table 1. Summary of Analytic Methods to Determine Polymorphisms

Gene	Polymorphism	PCR conditions	Primers
COMT	G to A transition in exon 4 that results in a *Val-Met* substitution and a lower enzymatic activity.	Buffer (10mM Tris-HCL (pH 8.3), 50 mM KCL), 1.5 mM MgCl2, 200 μM 2'-deoxynucleoside-3'-triphosphate, 1 unit of Taq DNA polymerase and primers. Total reaction volume of 30 μl using 300 ng of sample DNA. Denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 0.5 min, annealing at 60°C for 0.5 min, extension at 72°C for 0.5 min, and final annealing and extension steps at 72°C for 10 min. Digestion with NlaIII (New England Biolabs). Fragment sizes of 114, 54, 42 and 27 bp for the high-activity allele and 96, 54, 42, 27 and 18 bp for the low-activity allele.	5'-TACTGTGGCTACTCAGCTGTGC-3' and 5'-GTGAACGTGGTGTAACACC-3'
CYP1A1	1. T to C transition 264 bp downstream from the polyadenylate signal, a *Msp*I restriction site.	1. Amplification with primers in thermal cycler using 200 ng of DNA at 95°C for 4 min, 30 cycles with denaturation at 95°C for 1 min, annealing at 65°C for 1 min, and extension at 72°C for 1 min, final annealing and extension at 65°C for 1 min and 72°C for 8 min. Digestion with *Msp*I.	1. 5'-TAGGAGTCTTTGTCTCATGCCT-3' and 5'-CAGTGAAGAGGTGTAGCCGCT-3'
	2. A to G transition in exon 7 results in the substitution of valine for isoleucine at residue 462.	2. Initial denaturation at 94°C for 1.5 min, 25 cycles with denaturation at 94°C for 1 min, annealing and extension at 70°C for 1.5 min.	2. 5'-AAGACCTCCCAGCGGGCAAT-3' and 5'-AAGACCTCCCAGCGGGCAAC-3'
CYP1A2	At intron 1, 734 bp downstream of the first transcribed nucleotide of the gene.	PCR reaction: 94°C for 4'; 94°C for 30"; 60°C for 10"; 72°C for 1' X 35 cycles; and 72°C for 7'. The PCR product is 920 bop in size and is cleaved by Bsp120I to 709 and 211 bp fragments if the mutation is present and uncleaved if wildtype.	5'-CAACCCTGCCAATCTCAAGCAC-3' and 5'-AGAAGCTCTGTGGCCGAGAAGG-3'
CYP1B1	C→G transversion position 1666 of cDNA which changes amino acid 432 from Leu to Val.	Amplification of 0.2 μg genomic DNA through 30 cycles in a 100 μl volume containing 10 mM Tris-HCL (pH 8.3), 50 mM KCl, 4 mM MgCl₂, 200 μM each of the four deoxyribonucleotides, *Taq* polymerase (2 units) and each oligonucleotide at 25 μM. Denaturation at 95°C for 1 min, annealing at 62°C for 1 min and polymerization at 72°C for 1 min. Digestion with *Eco571* reveals the m1 polymorphism with bands of 104 and 39 bp, whereas digestion with *Cac81* identifies the m2 polymorphism with bands at 105 and 38 bp.	5'-GTGGTTTTTGTCAACCAGTGG-3' and 5'-GCGTCTTGCTTCTTATTGGCA-3'
CYP17	Sp1-type (CCACC box) promoter site 34 bp upstream from the initiation of translation, but downstream from the transcription start site.	PCR reactions in 25-μl aliquots containing about 50 ng of DNA, 50 pmol of each primer, 1xreaction buffer, 100μM deoxynucleotide triphophates, 1 unit of *Taq* polymerase. Amplification for 30 cycles with denaturation of 94°C for 1 min, annealing at 57°C for 1 min., and extension at 72°C for min. Digestion for 3h at 37°C using *Msp*AI.	5'-CATTCGCACCTCTGGAGTC-3' and 5'-GGCTCTTGGGGTACTTG-3'

Table 2. Percent Mammographic Density by Descriptive Factors

Descriptive characteristics	N (%)	Percent mammographic density	
		Means	Least-squares means *
Ethnicity			
Caucasian	118 (36)	35.23	34.92
Chinese	30 (9)	51.33	46.85
Filipino	13 (4)	43.51	37.88
Hawaiian	37 (11)	42.00	46.68
Japanese	90 (27)	42.11	39.74
Mixed	13 (4)	44.37	34.40
Other	27 (8)	36.76	32.12
p for GLM		p= 0.0217	p=0.0062
Ethnic category			
Caucasian	118 (36)	35.23	32.53
Asian	133 (41)	44.33	38.76
mixed and other	77 (23)	40.56	38.05
p for GLM		p=0.0071	p=0.0380
Menopausal status			
premenopausal	268 (82)	42.34	39.16
postmenopausal	60 (18)	30.45	34.56
p for GLM		p=0.0003	p=0.2877
Age			
<=40	55 (17)	39.46	37.11
41-45	149 (45)	43.48	42.04
46-50	65 (20)	44.83	41.19
51-55	21 (6)	25.44	20.72
56-60	12 (4)	25.68	25.30
>60	26 (8)	29.62	23.78
p for GLM		p=0.0001	p=0.0023
BMI			
<18.5	106 (33)	48.10	46.32
18.5-24.9	92 (28)	46.93	43.36
25-29.9	76 (24)	33.36	29.36
>=30	50 (15)	22.79	18.64
p for GLM		p< 0. 0001	p< 0.0001
Parity			
less than 3	234 (73)	42.37	31.72
3 or more	88 (27)	34.20	25.44
p for GLM		p=0.0042	p=0.0461
Age at menarche			
younger than 13	196 (61)	39.14	28.81
13 or older	126 (39)	41.77	28.35
p for GLM		p=0.3203	p=0.6994
Age at first live birth			
Under 30 years	166 (52)	37.31	29.37
30 and older	156 (48)	43.21	27.79
p for GLM		p=0.0217	p=0.5835
History of breast cancer in 1ˢᵗ degree relatives			
Yes	48 (15)	36.33	26.92
no	278 (85)	40.84	30.34
p for GLM		p=0.2101	p=0.3427

*Adjusted for all studied predictors of the mammographic density (see 'Methods' section)

Table 3. Frequency distribution of genotypes in different ethnic groups

Genes	Type	Total N (%)	Caucasian N (%)	Japanese N (%)	Hawaiian N (%)	Chinese N (%)	Filipino N (%)	Mixed N (%)	Other N (%)	x^2 (p)
COMT										
Val/Val=Wildtype	Val/Val	129 (39.45)	20 (16.95)	44 (49.44)	26 (70.27)	14 (46.67)	8 (61.54)	7 (53.85)	10 (37.04)	62.10 (<0.0001)
Val/Met= Heterozygote	Val/Met	140 (42.81)	60 (50.85)	33 (37.08)	10 (27.03)	25 (50.00)	5 (38.46)	6 (46.15)	11 (40.74)	
Met/Met=Mutant	Met/Met	58 (17.74)	38 (32.20)	12 (13.48)	1 (2.70)	1 (3.33)	0 (0.00)	0 (0.00)	6 (22.22)	
Cyp1A1-Msp										
A=Wildtype	A	165 (50.61)	86 (73.50)	30 (33.71)	13 (35.14)	9 (30.00)	3 (23.38)	6 (46.15)	18 (66.67)	65.14 (<0.0001)
B=Heterozygote	B	121 (37.12)	29 (24.79)	39 (43.82)	14 (37.84)	18 (60.00)	9 (69.23)	5 (38.46)	7 (25.93)	
C=Mutant	C	40 (12.27)	2 (1.71)	20 (22.47)	10 (27.03)	3 (10.00)	1 (7.69)	2 (15.38)	2 (7.41)	
Cyp1A1-exon7-BsrDI										
AA=Wildtype	AA	216 (66.06)	100 (84.75)	45 (50.56)	22 (59.46)	15 (50.00)	8 (61.54)	6 (46.15)	20 (74.07)	41.46 (<0.0001)
AG=Heterozygote	AG	95 (29.05)	17 (14.41)	34 (38.20)	14 (37.84)	14 (46.67)	4 (30.77)	6 (46.15)	6 (22.22)	
GG=Mutant	GG	16 (4.89)	1 (0.85)	10 (11.24)	1 (2.70)	1 (3.33)	1 (7.69)	1 (7.69)	1 (3.70)	
Cyp1A1 - exon 7 – RsaI										
CC=Wildtype	AA	310 (94.51)	108 (91.53)	87 (96.67)	33 (89.19)	29 (96.67)	13 (100.0)	13 (100.0)	27 (100.0)	14.93 (0.03)
CA=Heterozygote	CA	17 (5.18)	10 (8.47)	3 (3.33)	3 (8.11)	1 (3.33)	0 (0.00)	0 (0.00)	0 (0.00)	
AA=Mutant	CC	1 (0.30)	0 (0.00)	0 (0.00)	1 (2.70)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	
Cyp1A2-Intron1										
W=Wildtype	W	173 (52.91)	64 (54.24)	47 (52.81)	24 (64.86)	11 (36.67)	8 (61.54)	7 (53.85)	12 (44.44)	17.52 (0.13)
H=Heterozygote	H	131 (40.06)	48 (40.68)	35 (39.33)	9 (24.32)	18 (60.00)	5 (38.46)	6 (46.15)	10 (37.04)	
M=Mutant	M	23 (7.03)	6 (5.08)	7 (7.87)	4 (10.81)	1 (3.33)	0 (0.00)	0 (0.00)	5 (18.52)	
Cyp1B1-Pst										
LL=Wildtype	LL	143 (43.73)	31 (26.27)	54 (60.67)	14 (37.84)	21 (70.00)	9 (69.23)	6 (46.15)	8 (29.63)	48.69 (<0.0001)
LV=Heterozygote	LV	152 (46.48)	65 (55.08)	30 (33.71)	20 (54.05)	9 (30.00)	4 (30.77)	7 (53.85)	17 (62.96)	
VV=Mutant	VV	32 (9.79)	22 (18.64)	5 (5.62)	3 (8.11)	0 (0.00)	0 (0.00)	0 (0.00)	2 (7.41)	
Cyp17-MspA1										
W=Wildtype	W	113 (34.45)	47 (39.83)	30 (33.33)	13 (35.14)	5 (16.67)	3 (23.08)	6 (46.15)	9 (33.33)	15.95 (0.19)
H=Heterozygote	H	158 (48.17)	59 (50.00)	44 (48.89)	18 (48.65)	15 (50.00)	6 (46.15)	5 (38.46)	11 (40.74)	
M=Mutant	M	57 (17.38)	12 (10.17)	16 (17.78)	6 (16.22)	10 (33.33)	4 (30.77)	2 (15.38)	7 (25.93)	

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Table 3a. Frequency distribution of genotypes in different ethnic groups

Genes	Type	Total N (%)	Caucasian N (%)	Asian N (%)	Mixed N (%)	χ^2 (p)
COMT Val/Val=Wildtype Val/Met= Heterozygote Met/Met=Mutant	Val/Val	129 (39.5)	20 (16.95)	66 (50.00)	43 (55.840	48.99 (<0.0001)
	Val/Met	140 (42.8)	60 (50.85)	53 (40.15)	27 (35.06)	
	Met/Met	58 (17.7)	38 (32.20)	13 (9.85)	7 (9.09)	
Cyp1A1-Msp A=Wildtype B=Heterozygote C=Mutant	A	165 (50.6)	86 (73.50)	42 (31.82)	37 (48.05)	48.94 (<0.0001)
	B	121 (37.1)	29 (24.79)	66 (50.00)	26 (33.77)	
	C	40 (12.3)	2 (1.71)	24 (18.18)	14 (18.18)	
Cyp1A1-exon7-BsrDI AA=Wildtype AG=Heterozygote GG=Mutant	AA	216 (66.1)	100 (84.75)	68 (51.52)	48 (62.34)	33.64 (<0.0001)
	AG	95 (29.0)	17 (14.41)	52 (39.39)	26 (33.77)	
	GG	16 (4.9)	1 (0.85)	12 (9.09)	3 (3.90)	
Cyp1A1 - exon 7 – RsaI CC=Wildtype CA=Heterozygote AA=Mutant	AA	310 (94.5)	108 (91.53)	129 (96.99)	73 (94.81)	7.39 (0.1169)
	CA	17 (5.2)	10 (8.47)	4 (3.01)	3 (3.90)	
	CC	1 (0.3)	0 (0.00)	0 (0.00)	1 (1.30)	
Cyp1A2-Intron1 W=Wildtype H=Heterozygote M=Mutant	W	173 (52.9)	64 (54.24)	66 (50.00)	43 (55.84)	5.18 (0.2695)
	H	131 (40.1)	48 (40.68)	58 (43.94)	25 (32.47)	
	M	23 (7.0)	6 (5.08)	8 (6.06)	9 (11.69)	
Cyp1B1-Pst LL=Wildtype LV=Heterozygote VV=Mutant	LL	143 (43.7)	31 (26.27)	84 (63.64)	28 (36.36)	45.56 (<0.0001)
	LV	152 (46.5)	65 (55.08)	43 (32.58)	44 (57.14)	
	VV	32 (9.8)	22 (18.64)	5 (3.79)	5 (6.49)	
Cyp17-MspA1 W=Wildtype H=Heterozygote M=Mutant	W	113 (34.5)	47 (39.83)	38 (28.57)	28 (36.36)	8.54 (0.073)
	H	158 (48.1)	59 (50.00)	65 (48.87)	34 (44.160	
	M	57 (17.4)	12 (10.17)	30 (22.56)	15 (19.48)	

Table 4. Odds of having heterozygous or mutant genotype vs. wild type among ethnic groups compared with Caucasians (OR=1.0, reference)

Polymorphisms	Type	Japanese		Hawaiian		Chinese		Filipino		Mixed		Other	
		N	OR (95%CI)	N	OR (95%CI)	N	OR (95%CI)	N	OR (95%CI)	N	OR (95%CI)	N	OR (95%CI)
COMT	HH	44	1.0 (reference)	26	1.0(reference)	14	1.0 (reference)	8	1.0 (reference)	7	1.0 (reference)	10	1.0 (reference)
LL= Mutant	HL	33	0.25(0.13-0.49)	10	0.13(0.05-0.31)	25	0.36 (0.15-0.87)	5	0.21 (0.06-0.71)	7	0.29 (0.09-0.95)	11	0.37 (0.14-0.99)
HH=Wildtype	LL	12	0.14 (0.06-0.33)	1	0.02 (0.01-0.16)	1	0.04 (0.01-0.31)	0	-	0	-	6	0.32 (0.01-0.99)
HL= Heterozygote NW= HL+LL	NW	45	0.21(0.11-0.39)	11	0.09 (0.04-0.20)	26	0.23 (0.10-0.55)	5	0.13 (0.04-0.43)	7	0.18 (0.05-0.58)	17	0.35 (0.14-0.87)
Cyp1A1-Msp	A	30	1.0 (reference)	13	1.0 (reference)	9	1.0 (reference)	3	1.0 (reference)	6	1.0 (reference)	18	1.0 (reference)
A=Wildtype	B	29	3.9 (2.0-7.3)	14	3.2 (1.3-7.6)	18	5.9 (2.4-14.7)	9	8.9 (2.3-35.1)	5	2.47 (0.7-8.7)	7	1.15 (0.4-3.0)
B=Heterozygote C=Mutant	C	20	28.7 (6.3-130.0)	10	33.1 (6.5-168.2)	3	14.3 (2.1-97.4)	1	14.3 (1.0-205.4)	2	14.3 (1.7-120.3)	2	4.8 (0.6-36.2)
NW=B+C	NW	49	5.4 (3.00-9.9)	24	5.1 (2.3-11.3)	21	6.4 (2.7-15.6)	10	9.2 (2.4-35.8)	7	3.2 (1.0-10.3)	9	1.4 (0.6-3.4)
Cyp1A1-exon7-BsrDI	AA	45	1.0 (reference)	22	1.0 (reference)	15	1.0 (reference)	8	1.0 (reference)	6	1.0 (reference)	20	1.0 (reference)
AA=Wildtype	AG	34	4.4 (2.3-8.8)	14	3.7 (1.6-8.7)	14	5.5 (2.3-13.4)	4	2.9 (0.8-10.9)	6	5.9 (1.7-20.4)	6	1.8(0.6-5.1)
AG=Heterozygote GG=Mutant	GG	10	22.2(2.8-178.7)	1	4.5 (0.3-75.4)	1	6.7 (0.4-112.2)	1	12.5 (0.7-218.9)	1	16.7 (0.9-300.1)	1	5.0 (0.3-83.2)
NW=AG+GG	NW	44	5.4 (2.8-10.4)	15	3.8 (1.7-8.7)	15	5.6 (2.3-13.3)	5	3.4 (1.02-11.81)	7	6.5 (1.9-21.5)	7	1.9 (0.7-5.3)
Cyp1A1-exon7-Rsa*	CC	87	1.0 (reference)	33	1.0 (reference)	29	1.0 (reference)	13	1.0 (reference)	13	1.0 (reference)	27	1.0 (reference)
CC=Wildtype	CA	3	0.4 (0.1-1.4)	3	1.0 (0.3-3.8)	1	0.4 (0.1-3.03)	0	-	0	-	0	-
CA=Heterozygote	AA	0	-	1	-	0	-	0	-	0	-	0	-
AA=Mutant NW=CA+AA	NW	3	0.37 (0.10-1.40)	4	1.30(0.39-4.45)	1	0.37 (0.5-3.03)	0	-	0	-	0	-
Cyp1A2-Intron1	W	47	1.0 (reference)	24	1.0 (reference)	11	1.0 (reference)	8	1.0 (reference)	7	1.0 (reference)	12	1.0 (reference)
W=Wildtype	H	35	1.0 (0.6-1.8)	9	0.5 (0.2-1.2)	18	2.2 (0.9-5.0)	5	0.8 (0.3-2.7)	6	1.1 (0.4-3.6)	10	1.1(0.4-2.8)
H=Heterozygote M=Mutant	M	7	1.6 (0.5-5.0)	4	1.8 (0.5-6.9)	1	1.0 (0.1-8.9)	0	-	0	-	5	4.4 (1.2-16.9)
NW=H+M	NW	42	1.06 (0.61-1.84)	13	0.64 (0.30-1.39)	19	2.05 (0.87-4.68)	5	0.74(0.23-2.40)	13	1.02 (0.64-3.44)	15	1.48 (0.64-3.44)

* No homozygous carriers of the mutant allele among Caucasians (reference group).

To be continued on the next page

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Table 4. Continued

Cyp1B1-Pst													
LL=Wildtype	LL	54	1.0 (reference)	14	1.0 (reference)	21	1.0 (reference)	9	1.0 (reference)	6	1.0 (reference)	8	1.0 (reference)
LV=Heterozygote	LV	30	0.3 (0.1-0.5)	20	0.7 (0.3-1.5)	9	0.2 (0.1-0.5)	4	0.2 (0.1-0.7)	7	0.6 (0.2-1.8)	17	1.0 (0.4-2.6)
VV=Mutant	VV	5	0.1 (0.1-0.4)	1	0.3(0.1-1.2)	0	-	0	-	0	-	2	0.4 (0.1-1.8)
NW=LV+VV	NW	35	0.23 (0.13-0.42)	21	0.59 (0.27-1.28)	9	0.15 (0.6-0.37)	4	0.16 (0.05-0.55)	7	0.42 (0.13-1.33)	19	0.85 (0.34-2.13)
Cyp17-MspA1	W	30	1.0 (reference)	13	1.0 (reference)	5	1.0 (reference)	3	1.0 (reference)	6	1.0 (reference)	9	1.0 (reference)
W=Wildtype	H	44	1.2 (0.6-2.1)	18	1.1 (0.5-2.5)	15	2.4 (0.8-7.1)	6	1.6 (0.4-6.7)	5	0.7 (0.2-2.3)	11	1.0 (0.4-2.6)
H=Heterozygote	M	16	2.1 (0.9-5.0)	6	1.8 (0.6-5.7)	10	7.8 (2.5-27.2)	4	5.2 (1.0-26.5)	2	1.3 (0.2-7.3)	7	3.0 (0.9-9.9)
M=Mutant													
NW=H+M	NW	60	1.32 (0.75-2.35)	24	1.22 (0.57-2.64)	25	3.31(91.18-9.26)	10	2.21 (0.60-8.44)	7	0.77 (0.24-2.44)	18	1.32 (0.55-3.20)

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Table 5. Mammographic percent density by genotypes

Polymorphism	Type	N	Mean percent density	P for ANOVA	Least-squares mean percent density*	P for GLM
COMT Val/Val = Wildtype Va/Met = Heterozygote Met/Met=Mutant	**Val/Val**	129	43.66		41.12	
	Val/Met	140	38.99		38.76	
	Met/Met	58	34.79	**0. 04**	32.56	**0. 03**
Cyp17-MspA1 A1/A1=Wildtype A1/A2=Heterozygote A2/A2=Mutant	**A1/A1**	113	38.13		35.95	
	A1/A2	158	41.18		38.68	
	A2/A2	57	41.39	0.51	41.52	0.21
Cyp1B1-Pst LL=Wildtype LV=Heterozygote VV=Mutant	**LL**	143	43.47		40.82	
	LV	152	37.45		36.22	
	VV	32	37.46	0.06	41.29	0.11
Cyp1A1 - exon7 - BsrDI AA=Wildtype AG=Heterozygote GG=Mutant	**AA**	216	38.30		36.54	
	AG	95	41.98		38.60	
	GG	16	52.85	**0.03**	44.87	0.23
Cyp1A1 - exon 7 – Rsa CC=Wildtype CA=Heterozygote AA=Mutant	**CC**	310	40.35		37.39	
	CA	17	36.71		37.61	
	AA	1	43.60	0.81	49.77	0.82
Cyp1A1-Msp A=Wildtype B=Heterozygote C=Mutant	**A**	165	38.78		37.59	
	B	121	39.96		39.03	
	C	40	46.80	0.14	45.01	0.10
Cyp1A2 - intron 1 W=Wildtype H=Heterozygote M=Mutant	**W**	173	41.46		40.85	
	H	131	38.75		37.32	
	M	23	37.31	0.50	34.77	0.19

* Adjusted for age, BMI, age at menarche, parity, maternal age, menopausal status/HRT.

Table 6. Percent mammographic density by genotypes stratified on ethnic category

Polymorphisms	Type	Percent density by ethnic group								
		Caucasian			Asian			Mixed		
		N	D*	D**	N	D*	D**	N	D*	D**
COMT Val/Val=Wildtype Val/Met= Heterozygote Met/Met=Mutant	Val/Val	20	36.67	26.34	66	47.53	47.50	43	40.96	39.78
	Val/Met	60	36.05	29.23	53	40.99	42.80	27	41.58	39.74
	Met/Met	38	33.17	20.80	13	39.87	43.99	7	34.14	33.58
P for GLM			0.80	0.12		0.23	0.42		0.73	0.79
Cyp1A1-exon7-BsrDI AA=Wildtype AG=Heterozygote GG=Mutant	AA	100	35.89	26.69	68	**40.75**	45.44	48	39.86	38.52
	AG	17	31.15	22.98	52	**45.09**	45.19	26	42.87	39.73
	GG	1	38.50	31.14	12	**59.34**	57.72	3	31.67	28.72
P for GLM			0.73	0.76		**0.03**	0.13		0.68	0.69
Cyp17-MspA1 W=Wildtype H=Heterozygote M=Mutant	W	47	34.50	25.51	38	42.58	43.20	28	38.18	32.16
	H	59	37.04	26.39	65	44.94	46.16	34	41.21	38.60
	M	12	29.18	25.51	30	45.21	44.90	15	43.53	41.53
P for GLM			0.54	0.86		0.86	0.77		0.74	0.33
Cyp1B1-Pst LL=Wildtype LV=Heterozygote VV=Mutant	LL	31	**44.68**	**36.19**	84	44.24	44.48	28	39.86	33.96
	LV	65	**31.00**	**20.78**	43	43.91	46.08	44	40.65	37.02
	VV	22	**34.38**	**29.93**	5	44.84	51.94	5	43.66	43.85
P for GLM			**0.022**	**0.001**		0.99	0.68		0.94	0.64
Cyp1A1 - exon 7 – Rsa CC=Wildtype CA=Heterozygote AA=Mutant	AA	108	34.99	26.33	129	44.39	45.20	73	41.12	38.60
	CA	10	37.79	28.28	4	42.23	41.68	3	25.77	39.56
	CC	0	-	-	0	-	-	1	43.60	48.93
P for GLM			0.71	0.76		0.85	0.72		0.51	0.89
Cyp1A1-Msp A=Wildtype B=Heterozygote C=Mutant	A	86	37.27	28.27	42	42.72	45.54	37	37.82	34.61
	B	29	30.30	43.17	66	42.20	44.11	26	45.04	41.17
	C	2	34.90	51.25	24	52.02	51.95	14	39.48	35.99
P for GLM			0.37	0.48		0.17	0.25		0.45	0.49
Cyp1A2-Intron1 W=Wildtype H=Heterozygote M=Mutant	W	64	36.84	27.58	66	46.30	49.33	43	40.92	41.31
	H	48	34.10	25.70	58	41.61	42.93	25	41.07	39.16
	M	6	27.07	20.96	8	44.89	46.77	9	37.41	29.06
P for GLM			0.55	0.69		0.52	0.22		0.91	0.30

D*   percent density   D**   percent density adjusted for age, BMI, , parity, age at maternity, menopausal status/HRT.

Table 7. Characteristics of the Study Population for the Urinary Hormone Analysis

Variable	Asian	Mixed/ Others	Caucasian	All		p-value*
				Mean	Std	
Number	123	72	110	305		--
Menopausal	28%	18%	24%	25%		0.32
Age (years)	48.5	45.4	46.8	47.2	8.0	0.03
Body mass index (kg/m^2)	23.4	26.4	25.7	24.9	5.1	0.0004
Percent density	45.16	39.90	34.92	40.23	23.13	0.003
Estrone (ng/ml)	11.82	14.27	11.90	12.43	19.78	0.68
Estradiol (ng/ml)	3.90	4.85	3.84	4.11	5.03	0.43
2-OHestrone (ng/ml)	16.39	19.54	20.99	18.80	14.07	0.04
16α-OHestrone (ng/ml)	11.60	12.52	11.08	11.63	8.45	0.52
2/16α-OH ratio	1.56	1.81	2.02	1.79	1.00	0.0006
Testosterone (ng/ml)	2.83	4.06	4.16	3.60	2.94	0.0007
Androstanediol (ng/ml)	19.94	25.78	21.82	22.00	17.26	0.07
All hormones (ng/ml)	67.06	82.27	75.28	73.62	48.77	0.09
Estrogen/androgen ratio	2.71	2.23	2.73	2.61	3.41	0.50

* χ^2-test for categorical and ANOVA for continuous variables

Table 8. Relation of Hormones with Percent Densities, Age, and Body Mass Index

Variable	Spearman Correlation Coefficients (p) with			
	Age	BMI	Percent density	
			Unadjusted	Adjusted*
Age	--	-0.09 0.11	-0.21 0.0003	--
Body mass index	-0.09 0.11	--	-0.42 <.0001	--
Estrone (ng/ml)	-0.14 0.02	0.05 0.42	0.10 0.10	0.07 0.23
Estradiol (ng/ml)	-0.17 0.004	0.11 0.07	0.05 0.41	0.02 0.72
2-OHestrone (ng/ml)	-0.32 <.0001	-0.04 0.51	0.13 0.02	0.02 0.70
16α-OHestrone (ng/ml)	-0.34 <.0001	0.07 0.23	0.06 0.30	-0.04 0.48
2/16α-OH ratio	-0.09 0.12	-0.07 0.25	0.09 0.13	0.07 0.22
Testosterone (ng/ml)	-0.20 0.0005	0.27 <.0001	-0.03 0.57	0.02 0.77
Androstanediol (ng/ml)	-0.39 <.0001	0.21 0.0002	0.04 0.49	0.005 0.92
All hormones (ng/ml)	-0.28 <.0001	0.09 0.11	0.08 0.14	0.03 0.62
Estrogen/androgen ratio	0.04 0.48	-0.19 0.0007	0.10 0.08	0.05 0.44

* Adjusted for age, menopausal status, hormone replacement therapy, body mass index, ethnicity, age at menarche, age at first live birth, number of children

Figure 1. Levels of Urinary Hormones by Categories of Percent Density

Figure 1. Levels of Urinary Hormones by Categories of Percent Density